REMARKS

In the Office Action dated June 9, 2009, claims 74-90, 92-95, 97-107, 109-112, 114 and 115 are pending, of which claims 74-89 are withdrawn from consideration as directed to non-elected subject matter, and claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected.

Specifically, claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to satisfy the written description requirement. Claims 90, 92-95, 97-107, 109-112 and 114-115 are also rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enabling support in the specification. Claims 90, 92-95, 104-107 and 109-112 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Martinet et al. (*Biotechnology Letters* 20: 1171-1177, 1998) in view of JP 8336387, Nakanishi-Shindo et al. (*J. Biol. Chem.* 268: 26338-26346, 1993) and Chiba et al. (*J. Biol. Chem.* 41: 26298-26304, 1998). Claims 97-106 are rejected under 35 U.S.C. §103(a) as unpatentable over Martinet et al. in view of JP 8336387, Nakanishi-Shindo et al. and Chiba et al. as applied to claims 90, 92-95, 104-107 and 109-112 above, and further in view of Trombetta et al. (*J. Biol. Chem.* 271: 27509-27516, 1996). Claims 90, 92-95, 105 and 114 are rejected on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 1-2, 4-6, 9 and 14 of the U.S. Patent No. 7,252,933, as well as over claims 1-20 of U.S. Patent No. 7,507,573. Further, 90, 92-95, 97-107, 109-112 and 114-115 are rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225.

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim Amendments

Independent claims 90 and 107 have been amended to further define the Och1 disruption as the sole genetic disruption of the Golgi mannosyl transferases acting in N-glycosylation of the strain, a feature previously delineated in dependent claims 114 and 115, respectively. Claims 114-115 have been cancelled.

Claims 90, 93, 107 and 110 have also been amended to replace the term "functional part" of *T. reesei* α-1,2-mannosidase with "enzymatically active fragment". Support for this amendment is found in the specification, both implicitly and explicitly, e.g., on page 15, lines 15-27 of the specification (including a reference to "a catalytic domain").

No new matter is introduced by the foregoing amendments.

35 U.S.C. §112, First Paragraph (Written Description)

Claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to satisfy the written description requirement.

According to the Examiner, the instant disclosure fails to provide sufficient written description for a representative number of species for a broad genus of a functional part or a fragment of *T. reesei* α-1,2-mannosidase whose expression in the *Pichia* strain results in the specific desired result as broadly claimed. The Examiner alleges that the instant specification fails to describe the essential core structure(s) element(s) possessed by any functional part or any fragment of *T. reesei* α-1,2-mannosidase so that the functional fragment is sufficiently stable and active in the *Pichia* strain to yield the specific desired activity as claimed. As defined by the instant specification, the term "functional part" is meant to be a polypeptide fragment of an α-1,2-mannosidase which substantially retains the enzymatic activity of the full-length protein (page

15, lines 15-17). The Examiner states that at the effective filing date of the present application (6/30/2000), the absolute enzymatic activity of *T. reesei* α-1,2-mannosidase is not correlated with the production of a predominant N-glycan structure or a predominant intermediate N-glycan structure, as evidenced at least by the teachings of Martinet et al. (*Biotechnology Letters* 20: 1171-1177, 1998) and Callewaert et al. (*FEBS Letters* 503: 173-178, 2001). Moreover, the Examiner refers to Choi et al. (*PNAS* 100: 5022-5027, 2003), which allegedly also teaches that a proper length of the α-1,2-mannosidase catalytic domain is one of several factors that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains (see at least page 5026, col. 1, second full paragraph).

Applicants respectfully disagree.

In the first instance, Applicants respectfully submit that the art relied on by the Examiner does not support the Examiner's conclusion that the absolute enzymatic activity of *T. reesei* α-1,2-mannosidase is not correlated with the production of a predominant N-glycan structure or a predominant intermediate N-glycan structure.

With respect to Martinet et al., this reference describes a *Pichia pastoris* strain transformed to express a full length *T. reesei* α-1,2 mannosidase or a fusion of the catalytic domain of *T. reesei* α-1,2 mannosidase with the ER retention signal of *S. cerevisiae* MNS1. Yet the transformed strain failed to produce $Man_5GlcNAc_2$. The authors discussed several possibilities that may explain the failure to produce $Man_5GlcNAc_2$, including possible hyperglycosylation and the presence of capping glucose residues and phosphate groups. There is no evidence in this reference that would indicate that the failure to produce $Man_5GlcNAc_2$ resulted from the use of an inactive fragment of *T. reesei* α-1,2-mannosidase. On the contrary, the observation by the authors of a very clear effect (hyperglycosylation of the HA expressed)

that was dependent upon expression of the *T. reesei* α-1,2-mannosidase catalytic domain, argues strongly that this fragment of the enzyme retains activity.

Regarding Callewaert et al., this reference shows that the smallest sugar chain in the N-glycan pattern of the HA co-expressed with *T. reesei* α-1,2-mannosidase was a $Man_6GlcNAc_2$ isomer, instead of $Man_5GlcNAc_2$. Again, there is no evidence in this reference that would indicate that the failure to produce $Man_5GlcNAc_2$ resulted from an inactive fragment of *T. reesei* α-1,2-mannosidase. In hindsight, this failure may very well be a result of the activity of α-1,6-mannosyltransferase encoded by OCH1.

Turning to Choi et al., the authors stated that a proper length of the α-1,2-mannosidase catalytic domain is one of several factors that determine the yield of $Man_5GlcNAc_2$ in *P. pastoris* Och1 mutant strains. However, there was no actual data showing the differences in length of the "catalytic domains" employed, or the differences (or at least the extent of differences" in the yield of $Man_5GlcNAc_2$. Further, *T. reesei* α-1,2-mannosidase was not among the enzymes being investigated in these experiments.

Therefore, Applicants respectfully submit that the art at the relevant time does not lead one to conclude that an enzymatically active fragment of *T. reesei* α-1,2-mannosidase, such as a fragment containing the catalytic domain, would not have sufficient activity to produce a predominant N-glycan structure or a predominant intermediate N-glycan structure.

In fact, Applicants respectfully submit that *T. reesei* α-1,2- mannosidase was an enzyme well characterized in the art at the time the present application was filed, and fragments of this enzyme that are expected to substantially retain the functional activity of the enzyme, such as its catalytic domain, were already described in the art or could be readily determined by those skilled in the art. As support of this position, Applicants provide herewith **Exhibit 1**, which

provides an alignment of amino acid sequences of several α-mannosidases (including *T. reesei* α-1,2- mannosidase), all of which were available at the time the present application was filed. Such sequence alignment reveals very clear homology among α-mannosidases, which permits an easy determination of the conserved catalytic domains of these proteins (shown by shaded area). Further, *in vitro* assays are available and described in the art which permit an easy comparison of the activity of a particular fragment of *T. reesei* α-1,2- mannosidase with that of the full-length protein. See, e.g., U.S. Patent 5,834,251, Examples 7-8 on colns. 24-25 **(Exhibit 2)**.

Applicants respectfully submit that those skilled in the art would readily ascertain enzymatically active fragments of *T. reesei* α-1,2- mannosidase, such as its catalytic domain, and would reasonably expect that such enzymatically active fragments of *T. reesei* α-1,2-mannosidase would be able to produce a predominant N-glycan structure or a predominant intermediate N-glycan structure in a strain as presently claimed. The Examiner has not provided any evidence that would indicate otherwise. In fact, the disclosure of Martinet et al. supports that the catalytic domain of *T. reesei* α-1,2- mannosidase employed therein was fully active in removing terminal α-1,2-mannose residues. See the last six lines on page 1175 and the first five lines on page 1176 of Martinet et al.

Therefore, Applicants respectfully submit that the presently claimed subject matter, insofar as it concerns enzymatically active fragments of *T. reesei* α-1,2- mannosidase, is adequately described in the specification in a manner that satisfies the written description requirement.

35 U.S.C. §112, First Paragraph (Enablement)

Claims 90, 92-95, 97-107, 109-112 and 114-115 are rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enabling support in the specification.

The Examiner contends that while the specification is enabling for a genetically engineered strain of *Pichia*, wherein said strain is transformed with a nucleotide sequence coding for a full-length *T. reesei* α-1,2-mannosidase, it does not reasonably provide enablement for a genetically engineered strain of *Pichia* transformed with other nucleotide sequences coding for a *T. reesei* α-1,2-mannosidase or functional part thereof to attain the specific desired result, as broadly claimed. In explaining the basis of the rejection, the Examiner again refers to Martinet et al., Callewaert et al., and Choi et al., as alleged evidence of unpredictability in the art.

The art may have collectively demonstrated the difficulties in producing $Man_5GlcNAc_2$ in *P. pastoris*. However, there is no indication in the cited art that the difficulties were a result of employing fragments of *T. reesei* α-1,2-mannosidase of varying lengths. As submitted above, those skilled in the art would readily ascertain enzymatically active fragments of *T. reesei* α-1,2- mannosidase, such as its catalytic domain, and would reasonably expect that such enzymatically active fragments of *T. reesei* α-1,2- mannosidase would be able to produce a predominant N-glycan structure or a predominant intermediate N-glycan structure in a strain as presently claimed. The Examiner has not provided any evidence that would indicate otherwise. The Rudinger reference relied on by the Examiner on page 8 of the Action is merely making a generalized statement regarding modification to a "critical structural region of a protein" such as a "conservative substitution". The enzymatically active fragments of *T. reesei* α-1,2-mannosidase do not include modifications to a "critical structural region of a protein" such as a "conservative substitution" in the catalytic domain.

Accordingly, Applicants respectfully submit that those skilled in the art would be able to practice the presently claimed subject matter using enzymatically active fragments of *T. reesei* α-1,2- mannosidase, without undue experimentation. Withdrawal of the enablement requirement is therefore respectfully requested.

35 U.S.C. §103

Applicants observe that Claims 114-115 were not included in the obviousness rejection. Further, the Examiner states that except for claims 114-115, which are not rejected as obvious, the rejected claims encompass the making and/or use of a genetically engineered strain of *Pichia* whose genome comprises disruption of genes such as Och1/Mnn1, Och1/Mnn1/alg3 or Och1/Mnn1/Mnn4, and is not necessarily limited to only the single disruption of the Och1 gene in the genome of a *Pichia* strain.

In an effort to favorably advance prosecution, independent claims 90 and 107 have been amended to incorporate the features delineated in claims 114 and 115, respectively. As such, the obviousness rejections are obviated and withdrawal thereof is respectfully requested. Applicants reserve the right to pursue the subject matter encompassed by the claims prior to the instant amendment.

Non-Statutory Double Patenting

Applicants acknowledge that the double patenting rejections can be overcome by filing a terminal disclaimer. Applicants will file appropriate terminal disclaimers once the Examiner determines the claims to be otherwise allowable.

Conclusion

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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Enc.: Exhibits 1-2